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Pluteus umbrosoides and *P. chrysaegis*, new records from China

Md. Iqbal Hosen [1*], Jiang Xu [1, 2], Xishen Liang [1, 3], Tai-Hui Li [1*]

[1] *State Key Laboratory of Applied Microbiology Southern China, Guangdong Provincial Key Laboratory of Microbial Culture Collection & Application, Guangdong Institute of Microbiology, Guangzhou 510070, China*

[2] *Vegetables Research Institute, Guangdong Academy of Agricultural Sciences, Key Laboratory of Guangdong Vegetables New Technology, Guangzhou 510640*

[3] *South China Agricultural University, Guangzhou 510642, China*

[*] Correspondence to: *iqbalpatho@gmail.com, mycolab@263.net*

Abstract—*Pluteus umbrosoides* and *P. chrysaegis* are reported as new records from China based on morphological observation and molecular evidence. *Pluteus umbrosoides* is characterized by a brown to dark brown squamulose pileus lacking an appendiculate pileus margin, a nearly glabrous stipe, ellipsoid basidiospores, predominantly capitate pleurocystidia, and a trichohymeniderm pileipellis. *Pluteus chrysaegis* is distinguished by its bright yellow pileus, subglobose to globose basidiospores, lageniform to fusoid cheilocystidia with long apical appendages, and abundant fusoid pleurocystidia. Morphologically and phylogenetically both species are members of *P.* section *Hispidoderma*. Morphological descriptions, illustrations, and line drawings of Chinese collections of the two species are provided.

Key words—phylogeny, *Pluteaceae*, taxonomy

Introduction

Pluteus Fr. is the most widespread, saprotrophic, non-ectomycorrhizal, and type genus of the fungal family *Pluteaceae* (Singer 1986, Banerjee & Sundberg 1995, Justo & al. 2011a,b). Approximately 300 species have been reported (Kirk & al. 2008). The traditional subdivision of the genus into three sections (*P.* sect. *Pluteus*, *P.* sect. *Celluloderma* Fayod, and *P.* sect.

Hispidoderma Fayod) is with some rearrangements supported by molecular phylogenetic studies, (Justo & al. 2011a,b; Menolli & al. 2010, 2015a,b). *Pluteus* sect. *Hispidoderma* accommodates those species with non-metuloid pleurocystidia and a pileipellis consisting of elongated, filamentous elements organized as a cutis, hymeniderm, or trichoderm (Menolli & al. 2010; Justo & al. 2011a,b, 2012; Vizzini & Ercole 2011; Lezzi & al. 2014).

Here we present two new records from China, identified by morphological and molecular data: *P. umbrosoides* (originally described from Siberia, Russia; Malysheva & al. 2016) and *P. chrysaegis* (originally described from Sri Lanka; Pegler 1986). The newly recorded species are compared with morphologically similar species of *Pluteus*, and their phylogenetic positions are assessed.

Material & methods

Morphological studies

Fungal specimens of *Pluteus* were collected from China. The examined specimens were deposited in the Fungal Herbarium of the Guangdong Institute of Microbiology, Guangzhou, China (GDGM). Macromorphological descriptions were based on the field notes and documented by photographs.

Micromorphological observations were made from the dried specimens. Water, 5% aqueous KOH (w/v), Congo Red (staining tissues), and Melzer's solution (to detect amyloidity in basidiospores and tissues) were used in rehydration and as mounting media. Basidiospore measurements are annotated with [n/m/p] to denote 'n' basidiospores from 'm' basidiomata of 'p' collections and dimensions given as (a–) b–c(–d), with 'b–c' representing at least 90% of the measured values and extreme values 'a' and 'd' enclosed in parentheses when necessary. Q = length/width ratio per basidiospore, Q_m = average of 'n' basidiospores, SD = standard deviation, with results presented as $Q_m \pm$ SD.

Molecular studies

Protocols for genomic DNA extraction, PCR amplification, and sequencing followed Hosen & al. (2013). The ITS1-F/ITS4 (White & al. 1990) primer pair was used to amplify the nuclear ribosomal internal transcribed spacer (ITS) region.

A total of 65 sequences (Fig. 1) representing *Pluteaceae* was retrieved from GenBank based on NCBI blast search results and published analyses (Justo & al. 2011a,b; Menolli & al. 2010, 2015a,b; Pradeep & al. 2012; Malysheva & al. 2016) and combined with five newly generated ITS sequences from Chinese *Pluteus* material. The ITS sequences were aligned in MAFFT v.6.8 (Katoh & al. 2005) with default settings and manually edited in BioEdit v.7.0.9 (Hall 1999). A Maximum Likelihood (ML) analysis was performed using RAxML v.7.2.6 (Stamatakis 2006). As RAxML supports only the GTR model of nucleotide substitution, the GTRGAMMAI model was used for phylogenetic analyses, and statistical support

values were obtained using nonparametric bootstrapping (BS) with 1000 replicates. *Volvariella bombycina* (Schaeff.) Singer was selected as outgroup.

Molecular phylogenetic results

The ITS sequences newly generated from Chinese *Pluteus* specimens were deposited in GenBank (MH059511–MH059514). In the aligned ITS dataset,

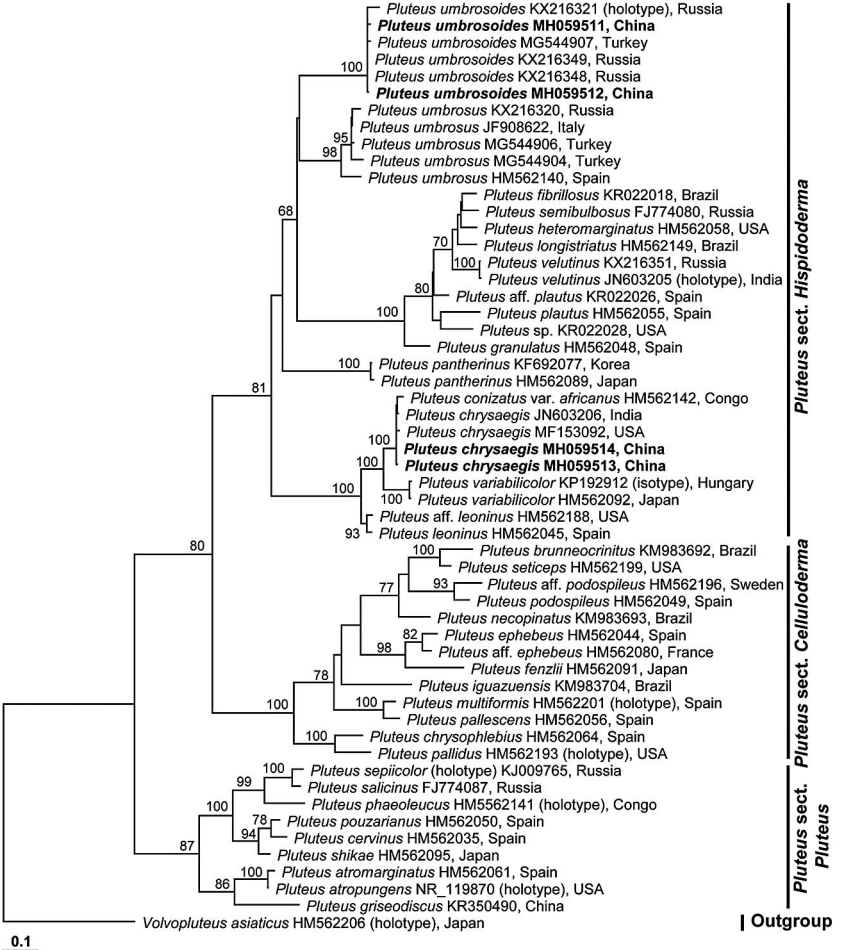
Pluteus umbrosoides KX216321 (holotype), Russia
Pluteus umbrosoides MH059511, China
Pluteus umbrosoides MG544907, Turkey
Pluteus umbrosoides KX216349, Russia
Pluteus umbrosoides KX216348, Russia
Pluteus umbrosoides MH059512, China
Pluteus umbrosus KX216320, Russia
Pluteus umbrosus JF908622, Italy
Pluteus umbrosus MG544906, Turkey
Pluteus umbrosus MG544904, Turkey
Pluteus umbrosus HM562140, Spain
Pluteus fibrillosus KR022018, Brazil
Pluteus semibulbosus FJ774080, Russia
Pluteus heteromarginatus HM562058, USA
Pluteus longistriatus HM562149, Brazil
Pluteus velutinus KX216351, Russia
Pluteus velutinus JN603205 (holotype), India
Pluteus aff. plautus KR022026, Spain
Pluteus plautus HM562055, Spain
Pluteus sp. KR022028, USA
Pluteus granulatus HM562048, Spain
Pluteus pantherinus KF692077, Korea
Pluteus pantherinus HM562089, Japan
Pluteus conizatus var. africanus HM562142, Congo
Pluteus chrysaegis JN603206, India
Pluteus chrysaegis MF153092, USA
Pluteus chrysaegis MH059514, China
Pluteus chrysaegis MH059513, China
Pluteus variabilicolor KP192912 (isotype), Hungary
Pluteus variabilicolor HM562092, Japan
Pluteus aff. leoninus HM562188, USA
Pluteus leoninus HM562045, Spain
Pluteus brunneocrinitus KM983692, Brazil
Pluteus seticeps HM562199, USA
Pluteus aff. podospileus HM562196, Sweden
Pluteus podospileus HM562049, Spain
Pluteus necopinatus KM983693, Brazil
Pluteus ephebeus HM562044, Spain
Pluteus aff. ephebeus HM562080, France
Pluteus fenzlii HM562091, Japan
Pluteus iguazuensis KM983704, Brazil
Pluteus multiformis HM562201 (holotype), Spain
Pluteus pallescens HM562056, Spain
Pluteus chrysophlebius HM562064, Spain
Pluteus pallidus HM562193 (holotype), USA
Pluteus sepiicolor (holotype) KJ009765, Russia
Pluteus salicinus FJ774087, Russia
Pluteus phaeoleucus HM5562141 (holotype), Congo
Pluteus pouzarianus HM562050, Spain
Pluteus cervinus HM562035, Spain
Pluteus shikae HM562095, Japan
Pluteus atromarginatus HM562061, Spain
Pluteus atropungens NR_119870 (holotype), USA
Pluteus griseodiscus KR350490, China
Volvopluteus asiaticus HM562206 (holotype), Japan
Pluteus sect. Hispidoderma
Pluteus sect. Celluloderma
Pluteus sect. Pluteus
Outgroup
0.1

Fig. 1. Phylogenetic relationships of *Pluteus* species and its allied genera inferred from ITS data using ML method. RAxML bootstrap support values ≥50% are indicated on the branches at nodes. Newly generated sequences of *Pluteus* species from China are set in bold font. GenBank accession numbers and country names are provided after the species name. The tree is rooted by *Volvariella bombycina* as the outgroup.

sequences of 55 sampled *Pluteaceae* specimens included 825 nucleotide sites (gaps included) per sample. Our phylogeny supports the Chinese sequences MH059511 and MH059512 as conspecific with *P. umbrosoides* (and closely related to *P. umbrosus* (Pers.) P. Kumm.), and sequences MH059513 and MH059514 as conspecific with *P. chrysaegis* (and closely related to *P. variabilicolor* Babos) (Fig. 1).

Fig. 2. ***Pluteus umbrosoides*** (GDGM 42350): a, b. Natural habit. ***Pluteus chrysaegis*** (GDGM 42287 and GDGM 42376): c. Natural habit (GDGM 42287): d. Natural habit. (GDGM 42376). Scale bars = 10 mm.

Taxonomy

Pluteus umbrosoides E.F. Malysheva, Mycol. Progr. 15: 880 (2016) Figs 2a,b; 3

Basidiomata small to medium-sized. Pileus 35–60 mm broad, hemispherical, convex to applanate with a slightly umbo, depressed in some areas, uneven; brown to dark brown, reddish brown to grayish brown, pale brown towards margin; surface covered by brown to dark brown or blackish brown squamules, more densely at centre; margin uneven, minutely serrate with undulate; pileus context 8 mm thick at centre, white, unchanged when injured. Lamellae 4 mm broad, free, crowded, slightly ventricose, pale pink to pink; lamellulae common at different lengths. Stipe 45–50 × 4–5 mm, cylindrical, slightly thickening towards base, smooth but minutely fibrillose nearly to the stipe base, elsewhere glabrous, pallid white to dull white, slightly

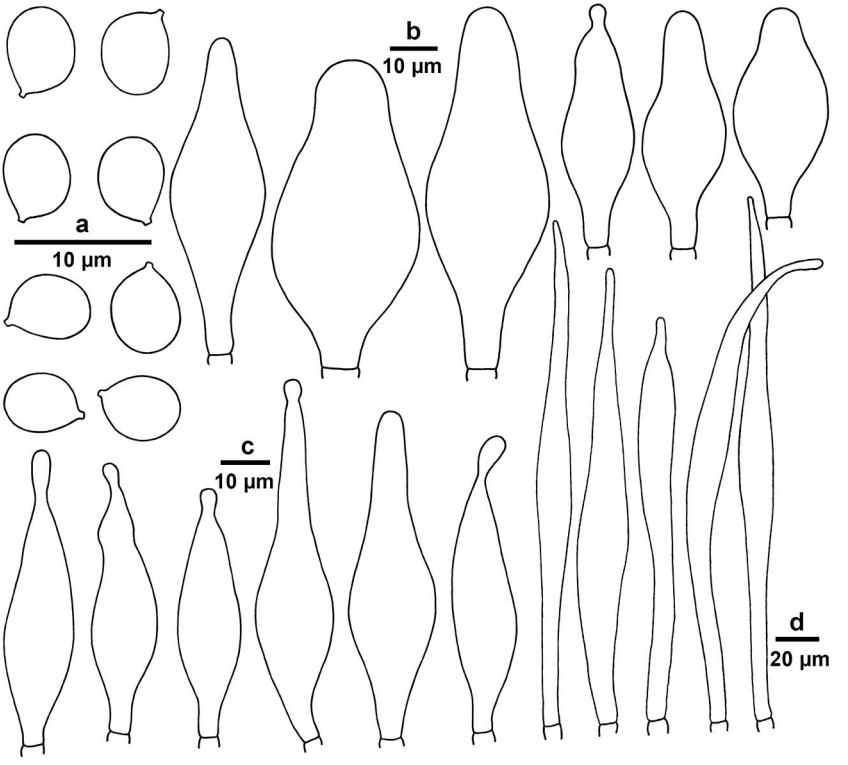

Fig. 3. ***Pluteus umbrosoides*** (GDGM 42350).
a. Basidiospores; b. Cheilocystidia; c. Pleurocystidia; d. Pileipellis elements.

darker at the base, solid to slightly fistulose; context white, unchanged when cut. Odor and taste not recorded.

Basidiospores [40/2/2] 5.5–6.5(–6.8) × (4.5–)5.0–6.0(–6.5) µm [mean length = 6.15, mean width = 5.23, Q = (1.05–)1.10–1.20(–1.23); Q_m = 1.17 ± 0.044), ellipsoid to subglobose, hyaline to pale yellow, thick-walled (0.5 µm thick). Basidia 20–26(–30) × 7–8 µm, 2–4-spored, clavate to narrowly clavate; sterigmata up to 3 µm long. Cheilocystidia 40–75 × 11–31 µm, abundant, fusiform to broadly fusiform, utriform, or broadly lageniform with appendage, sometimes capitulum head present, hyaline, thin-walled. Pleurocystidia 40–80 × 11–18 µm, abundant, fusiform to broadly fusiform, lageniform to broadly lageniform, tapering into apex generally bearing a capitulum head, sometimes also presenting cheilocystidium-like fusiform elements, hyaline, thin-walled.

Pileipellis a trichohymeniderm, composed of narrowly to broadly fusiform elements with tapering or obtuse or papillate apexes, 100–300 × 15–25 µm, with yellow-brown intracellular pigment, thin-walled. Stipitipellis vertically oriented, cylindrical hyphae 5–25 µm wide, pale yellow to yellowish; caulocystidia rare. Clamp connections absent in all tissues.

Specimens examined: **CHINA**, **Jilin province**: **Changjiang County**, Bawangling National Forest Park, Changbaishan Mountain, Lushui River, Deer Farm, 11 August 2011, leg. Ming Zhang, Xiao-Lan He, Hao Huang (GDGM29469, GenBank MH059512); 21 August 2012, leg. Jiang Xu (GDGM42350, GenBank MH059511).

Habitat & distribution—Solitary, grows on decayed wood. Recorded from Russia (Siberia) and China (and possibly Turkey, fide GenBank MG544907).

Pluteus chrysaegis (Berk. & Broome) Petch,
Ann. Roy. Bot. Gard. (Peradeniya) 5: 271 (1912) Figs 2c,d; 4

Basidiomata small to medium-sized. Pileus 25–45 mm broad, hemispherical, convex to plano-convex, wrinkled to rugulose in some areas, brilliant golden yellow to chrome yellow, central disc darker and often rugulose, margin finely striate, entire; context 3 mm thick at the pileus center, elsewhere thin, unchanged when cut or injured. Lamellae free, 5 mm broad, not ventricose, white then pale pink, crowded; lamellulae common with different lengths. Stipe 45–65 × 35–47 mm, central, cylindrical, slightly thickening towards the base, glabrous, surface pallid white to pale yellow, usually yellowish to brownish towards the base; basal mycelium whitish to yellowish white; context 2 mm thick, white, unchanged when cut. Taste and odor not recorded.

Basidiospores [40/2/2] 5.0–5.5(–6.2) × 4.5–5.0 µm [mean length = 5.28, mean width = 4.83, Q = (1.02–)1.07–1.15(–1.20); Qm = 1.09 ± 0.051], subglobose, globose to nearly broadly ellipsoid, hyaline to pale yellow, thick-walled (0.5 µm thick). Basidia 25–35 × 6–8 µm, 2–4-spored, clavate to narrowly clavate; sterigmata up to 3 µm long. Cheilocystidia 30–80 × 6–15 µm, abundant, fasciculate, mostly lageniform to fusiform with long appendage toward apex, hyaline, thin to slightly thick-walled. Pleurocystidia 45–75 × 10–20 µm, abundant, fusiform to broadly fusiform, rarely broadly clavate to mucronate, hyaline, thin-walled. Pileipellis an epithelial, intermixed with abundant pileocystidia, clavate cells measuring 15–25 × 6–10 µm, pileocystidia measuring 20–50 × 7–10 µm, similar to cheilocystidia but shorter and narrower, hyaline to pale yellow, thin-walled. Stipitipellis vertically oriented, cylindrical hyphae 4–10 µm wide, pale yellow to yellowish; caulocystidia not found. Clamp connections absent in all tissues.

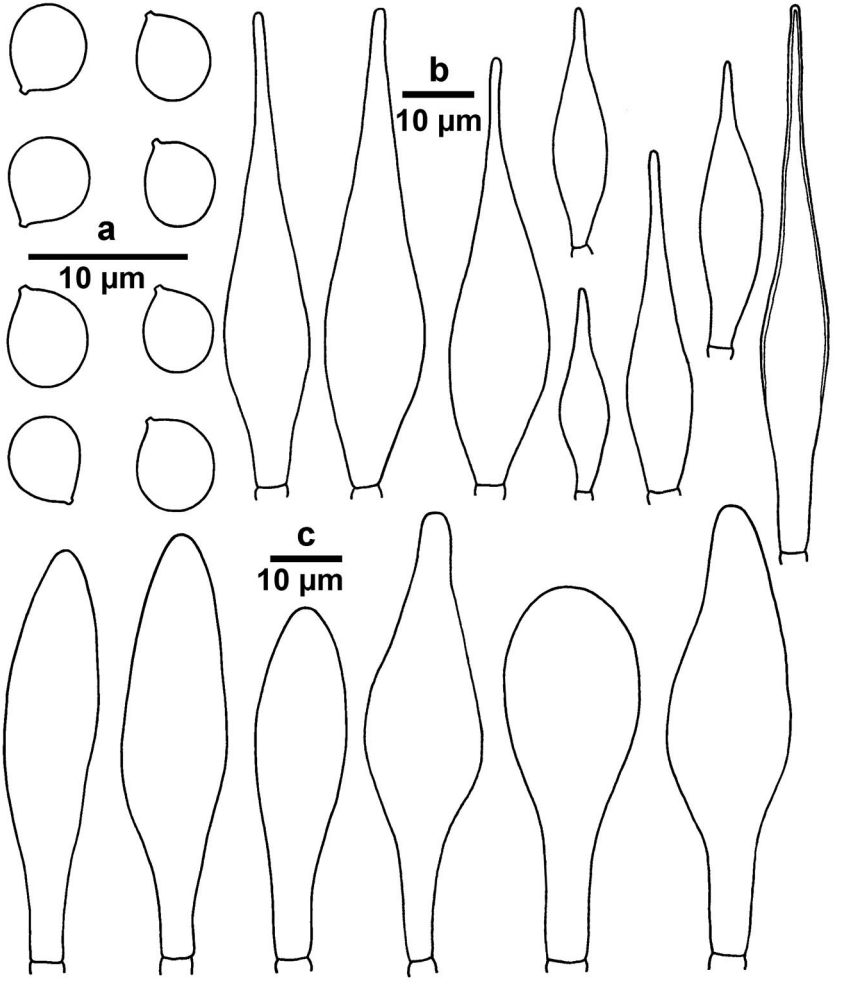

Fig. 4. ***Pluteus chrysaegis*** (GDGM 42287).
a. Basidiospores; b. Cheilocystidia; c. Pleurocystidia.

Specimens examined: **CHINA**, **Guangdong province**: **Shaoguan City**, Chebaling, Xianrendong National Nature Reserve Forest, 11 July 2013, leg. Jiang Xu, Bin Song, Ting Li and Wei Wang (GDGM42287, GenBank MH059513). **Hunan province: Chenzhou City**, Mangshan National Nature Reserve, 17 August 2014, leg. Xu Jiang, Zhou Shihao (GDGM42376, GenBank MH059514).

Habitat & distribution—Solitary, grows on decayed wood. Recorded from Sri Lanka, India, West Africa, and China (and possibly USA, fide GenBank MF153092).

Discussion

Pluteus umbrosoides, originally described from Russia (Krasnoyarsk, Siberia), is characterized by a brown to dark brown squamulose pileus lacking an appendiculate pileus margin and with squamules often arranged in a vein-like pattern, nearly smooth stipe, ellipsoid basidiospores, predominantly capitate pleurocystidia, and a trichohymeniderm pileipellis (Malysheva & al. 2016).

Our molecular phylogenetic analysis places the Chinese collections (GDGM42350 and GDGM29469) in *P.* sect. *Hispidoderma* and nested in the same lineage as the type material of *P. umbrosoides* (Fig. 1). Based on 620 nucleotides from the ITS sequence, genetic distances among the Russian collections (including type material), Turkish collection, and Chinese collections range from 0.32–0.82%, suggesting that all collections represent one species with intraspecific variation rather than being different species.

Pluteus umbrosoides is morphologically similar to *P. umbrosus* and *P. granularis* Peck. However, both of those species are distinguished from *P. umbrosoides* by their brown edged lamellae, a pileus margin that is distinctly appendiculate or covered with over-hanging floccules, non-smooth stipe surface, a stipe surface abundantly covered by caulocystidia, and longer pileipellis elements (Malysheva & al. 2016). Phylogenetically, *P. granularis* and *P. umbrosus* cluster together with strong BS support value and are separated from *P. umbrosoides* (Fig. 1). Geographically, *P. umbrosus* is distributed in Eurasia (Spain, Italy, Russia, and Turkey) and *P. granularis* is recorded only in USA, while *P. umbrosoides* is found in Russia (Siberia) and now China.

Pluteus chrysaegis, originally described from Sri Lanka, is characterized by a bright yellow pileus, subglobose to globose basidiospores, lageniform to fusoid cheilocystidia with long appendages, abundant fusiform pleurocystidia, and a pileipellis composed of clavate cells intermixed with fusiform pileocystidia (Pegler 1986, Pradeep & Vrinda 2006, Pradeep & al. 2012, Desjardin & Perry 2018). Initially, *P. chrysaegis* was placed in *P.* sect. *Celluloderma* based on the greater number of fusiform elements in the pileipellis (Pegler 1986, Pradeep & Vrinda 2006), but subsequent

molecular studies (Pradeep & al. 2012, Desjardin & Perry 2018, this paper) place it in *P.* sect. *Hispidoderma*.

Pluteus chrysaegis was known only from the type locality (Pegler 1986), until it was found in India (Pradeep & Vrinda 2006, Pradeep & al. 2012), West Africa (Desjardin & Perry 2018), and now China. One sequence (GenBank MF153092) from USA also clusters with *P. chrysaegis* (Fig. 1), suggesting that this species has a wide geographic distribution ranging from South Asia, East Asia, West and Equatorial Africa, and North America. *Pluteus conizatus* var. *africanus* E. Horak, originally described from Equatorial Africa (Horak 1977), nests in the same lineage and is considered to represent a synonym of *P. chrysaegis* (Pradeep & al. 2012, Desjardin & Perry 2018).

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